REMARKS

Claims 40-44 have been canceled as directed to a non-elected invention, and the subject matter of those claims will be pursued in a timely filed divisional application. The remaining claims have been amended, without prejudice to applicants' right to present claims of scope equivalent to those pending prior to this amendment in a timely filed continuing application. The limitations of claim 27 have been placed into claim 23; the wording has been consolidated in view of its inclusion in claim 23. Accordingly, claim 27 has been canceled and the dependency of claims 28-39 has been amended to depend on claim 23. Claims 28-39 have also been amended to clarify the order of modules, as requested. Claim 24 has been canceled. No new matter has been added and entry of the amendment is respectfully requested.

The Invention

As discussed at the interview, the invention arose, in part, from the finding that relatively non-conserved regions of polyketide synthases located between the C-terminus of the ACP catalytic domain in an upstream module and the N-terminus of the KS domain in an immediately downstream module to facilitate the transfer of the nascent polyketide chain from one module to the next. With the understanding that these linkers facilitate transfer, one method to obtain a modified polyketide is to manipulate entire modules in accordance with the methods of the invention, rather than, for example, inserting different catalytic domains into a module to change its specificity. Yields can be improved by taking the present approach of manipulating entire modules as opposed to single catalytic domains within the scaffolding of the module itself. As noted in the specification, the word "module" as used in the present claims does not include these intermodular linkers. See page 6 of the specification, lines 24-27. It is understood that in other contexts "module" may include at least portions of these linker sequences; however, for the purposes of the present invention, the discussion is facilitated by defining "module" as terminating at the ends of the included catalytic domains.

Formal Matters

Applicants appreciate the acknowledgement of benefit of priority of application 60/119,363 filed 9 February 1999. Priority is further discussed briefly below.

Applicants apologize for inconvenience to the Examiner of the plethora of documents submitted in regard to the Information Disclosure Statements submitted. Nonetheless, applicants believe the record must be complete and so submit herewith the copies of references that were missing from the existing statements as noted by the Office.

The objection to the drawings is noted, but form 948 was not found attached to the action. Therefore, applicants do not know what corrections are needed and respectfully request a copy of the form. A Request for Correction of Drawings was submitted 20 September 2001. A copy is enclosed.

The asserted incompleteness of the sequence listing is also noted; copies of papers to complete this requirement which were mailed 20 September 2001 are attached. The Preliminary Amendment contains instructions to enter on page 1, and verification of identity of the CRF and paper copy on page 7.

The objections to the specification are noted. The specification has been amended to insert the date of the Jacobsen, *et al.*, reference at page 15, line 6, of the specification, as well as to complete the citation in the succeeding paragraph..

A new abstract is submitted.

With respect to the general objection to the specification as "confusing," it appears that the Office is requesting explicit disclosure of material known in the art - *i.e.*, specifics concerning the sequences of various polyketide synthases that have been elucidated, published and are publicly available. As discussed with the Examiner in a telephone conversation following the interview, it has been established that those of skill in the art have ready access to these published sequences and that those sequences are part of the general knowledge of the art. As of the application date herein, those of skill in the art would understand that a multiplicity of modular PKS had been cloned and sequenced, that the catalytic domains of such PKS are readily recognizable, and that similar PKS with equally recognizable catalytic domains continue to be cloned and sequenced. Attached on pages 14-16 as Exhibit B, are summaries of junction regions between the C-terminus of the ACP domain and the N-terminus of the KS domain from several

different PKS's. As is evident from these summaries, the catalytic domain boundaries are highly homologous and readily recognizable by one of ordinary skill in the art. Exhibit B is a chapter from a thesis submitted to Stanford Universtiy by a student of Professor Khosla on 1 November 2000. The sequences are derived from a variety of literature sources, and are commonly available both in periodicals and in databases.

The Rejections Under 35 U.S.C. § 112, Paragraph 2

A portion of this rejection is based on the assertion that the intermodular linkers shown in Figure 3 lack substantial homology to each other. While the linkers do lack substantial homology, that lack is not a sound basis for this rejection. As discussed at the interview, the metes and bounds of the intermodular linkers are not defined by their similarity to each other, but rather by their location in a PKS. These linkers can be recognized as non-conserved regions located between the highly conserved catalytic domains. This aspect of the linkers is illustrated on pages 14-16 of Exhibit B, which shows that there is considerable lack of homology among the various linkers, but very strict homology at their boundaries, which are defined by the flanking catalytic domains. As to the specific linker between DEBS PKS modules 1 and 2, regardless of any designation in GenBank, the specification makes clear, as does Exhibit B, that the end of the RAL appended to the downstream KS of M2 is P-V-D; the typical pattern for the C-terminal end of the upstream ACP is also shown in Exhibit B.

Claims 25 and 26 have been modified as the Examiner suggests; as noted, Figure 3 shows only the N-terminal appended portions of the ERL. The number designation in all of the notations is with respect to the module downstream of the linker. This is apparent, for example, from the designation of the ERL which is consistently at the N-terminus of a separate molecule and also from those RAL where the position can be inferred also because this must be the case when the module is the C-terminal module of a distinct polypeptide, such as M2 *ery* and M4 *ery*.

All claims were rejected under this section as informal because of reference to "an" N-terminus and "a" C-terminus. This has been amended to conform to the Examiner's suggestion.

Claims 28-39 were rejected as indefinite as ambiguous as to whether modules 3-6 must be in the order 3-4-5-6, or that simply all present. Applicants believe that the terminology is clear; however, the claims have been amended to make the order explicit.

With respect to the criticism that claims 28-39 lack reference to loading modules, applicants believe this basis for rejection is in error. First, the claims are open and do not exclude the presence of loading modules; thus if a loading module is required, it would be understood to be present. In any event, as is evident from the specification, if, for example, a diketide starting material is employed, a "loading module" may not be required or desirable. It is believed that one of skill in the art would understand that, depending on the nature of the substrate, a loading module may or may not also be included in the hybrids described in these claims.

For the reasons set forth above, it is believed that the rejections under 35 U.S.C. § 112, paragraph 2, may be withdrawn.

The Rejection Under 35 U.S.C. § 112, Paragraph 1

All claims were rejected under this paragraph as putatively lacking written description. It is believed that this matter, as it relates to the extender modules, has been resolved. As discussed, the invention is not directed to the discovery of a particular sequence of amino acids or nucleotides; rather, the claimed composition is defined in terms of its hitherto unappreciated functional components that are already known and accessible to those of skill in the art, or become so upon determination of the amino acid sequence of the modular PKS. The claims are not drawn to claim intra-molecular or inter-molecular linkers as such. Rather, the claims are directed to hybrid PKS, constructs in which the advantage of supplying such linkers has been, for the first time, recognized by the current inventors.

As evidenced by the enclosed Declaration of Chaitan Khosla, one of skill in the art could readily identify the linkers in any naturally occurring PKS. Therefore, the written description in the specification is sufficient to permit one of skill in the art to practice the invention, and to

show that applicants had the invention as claimed in their possession when the application was filed.

Claims 28-39 were similarly rejected on the basis that the specific sequences represented by the designations in the claims are not provided directly in the specification. Respectfully, applicants believe that this basis for rejection is in error. As the Office concedes, the relevant sequences are already known in the art. As is established herein, one of ordinary skill can readily identify within these known sequences the modules referred to in these claims. The article by Dr. Khosla discussed at the interview, *Chem. Rev.* (1997) 97:2577-2580, clearly establishes the accuracy of this statement. The patent laws do not require applicants to spell out in the specification subject matter already known in the art. Thus, if one were to claim a pharmaceutical composition which is an inventive formulation of aspirin, it would not be necessary to put the chemical structure of aspirin into the specification. Or, if an invention resides in a synergistic effect between ibuprofen and acetyl choline, it would not be necessary to draw these structures in the specification. It might be helpful and convenient to a reviewer of the specification with less than ordinary skill in the pertinent art, but such inclusion of information known and readily available to those of skill in the art at the time an application is filed is not a requirement of the statute. Accordingly, this basis for rejection may be withdrawn.

The Rejections Over the Art

It is believed this rejection was resolved in favor of patentability of the claimed invention at the interview. However, for completeness, the following remarks are made.

Claims 23, 25, 26 and 27 were rejected as anticipated by McDaniel, *et al.* However, the claim limitations relating to the nature of the hybrid PKS of the instant claims are not met by this article. Further, there is no recognition that the appropriate linkers are necessary to assure nascent polyketide chain transfer. The McDaniel article, which is co-authored by one or more of the present inventors, has a different interpretation of the results presented; it is that "the amino and carboxyl termini of the PKS subunits are <u>not</u> required for transfer between covalent

modules." It is only the later work of this group, the basis for the present application, that resulted in the understanding that appropriate linkage is required.

Claims 23-25, and 27 were also rejected as anticipated by the Ranganathan document. As discussed, the assessment that the provisional application does not support the claims as presently drawn is no longer the view of the Office as the same examples appear in the priority document and instant application. Therefore, Ranganathan is not citable.

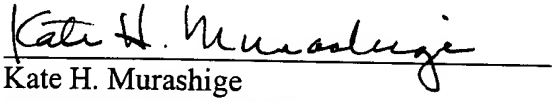
CONCLUSION

The claims have been amended to correct formal matters and to insert the limitations of claim 27 into claim 23. Various aspects of terminology have been clarified. It is believed that the rejections under 35 U.S.C. § 112, paragraph 1, may be withdrawn in light of the discussion at the interview and the nature of the invention. The cited art has been shown not to defeat patentability. Therefore, applicants respectfully request that all rejections be withdrawn and claims 23, 25-26 and 28-39 be passed to issue. If minor matters remain that might be resolved by discussion, a telephone call to the undersigned is respectfully requested.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. 300622004600.

Respectfully submitted,

Dated: August 12, 2002

By: 

Kate H. Murashige
Registration No. 29,959

Morrison & Foerster LLP
3811 Valley Centre Drive, Suite 500
San Diego, California 92130-2332
Telephone: (858) 720-5112
Facsimile: (858) 720-5125

EXHIBIT A. - VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Please amend the paragraphs on page 15, lines 5-18 as follows:

(M5+TE) was constructed by combining the engineered *NdeI* site from pJRJ10 (Jacobsen, J.R., *et al., Biochem* (19[___] 98) 37:4928-4934) with the *EcoRI* site from pCK15 (Cortes-Kao, *supra* [documents]). The *Nde-EcoRI* fragment was cloned in pET21c to obtain the expression plasmid pRSG46. Expression constructs for (M2+TE) and (M6+TE) were prepared similarly using an engineered *Nhe* site immediately upstream of the corresponding KS (at position 7570, 5′-GCTAGCGAGCCGATC-3′ and at position 28710, 5′-GCTAGCGACCCGATC-3′).

These constructs were expressed in *E. coli* BL21 (DE3) along with an expression system for *sfp* phosphopantetheinyl transferase from *B. subtilis*. The co-expression is described by Lambalot, R.H., *et al., Chem. Biol.* (1996) 3:923. For the construction of the *sfp* gene, the *NdeI-HindIII* fragment derived from the pUC8-*sfp* (Nakano, M.M., *et al.,* [*Mal.*] *Mol. Gen. Genet.* (19[___] 92) 232:313-321) was cloned into pET28 which has a kanamycin resistance gene to give resultant plasmid pRSG56. The resulting proteins were then isolated for use in the reaction mixtures described in the Examples below.

In the Claims:

23. (Amended) A hybrid modular polyketide synthase (PKS) comprising at least a first naturally occurring extender module and a second naturally occurring extender module of a different PKS from said first module,

wherein [a] the C-terminus of said first module is covalently linked to [an] the N-terminus of a naturally occurring intra-molecular linker (RAL) or inter-molecular linker (ERL) and [an] the N-terminus of the second module is covalently linked to [a] the C-terminus of said RAL or ERL, and

wherein either said first module or second module is not covalently linked to said RAL or ERL in a naturally occurring polyketide synthase;

whereby the transfer of a nascent polyketide chain from said first module to said second module is facilitated.

25. (Amended) The hybrid modular PKS of claim 23 wherein said RAL is selected from the group consisting of M2 *ery*, M4 *ery*, M6 *ery*, M2 *rif*, M3 *rif*, M5 *rif*, M3 *rap*, M4 *rap*, and M7 *rap* intra-module linkers (SEQ. ID. NO's: 3-11, respectively).

26. (Amended) The hybrid modular PKS of claim 23 wherein the ERL is selected from the group consisting of M3 *ery*, M5 *ery*, M4 *rif*, M7 *rif*, M8 *rif*, M9 *rif*, M5 *rap*, and M11 *rap* inter-module linkers wherein the portions of said modules coupled to the N-terminus of the succeeding module are represented by SEQ. ID. NO's: 12-19, respectively.

28. (Amended) The hybrid modular polyketide PKS of claim [27] 23 which contains *ery* modules 1[,] and [3-6] 3 through 6 inclusive and tylosin module 2, and wherein said polyketide chain is transferred from *ery* module 1 to *tyl* module 2 and then to *ery* modules [3-6] 3 through 6 inclusive.

29. (Amended) The hybrid modular polyketide PKS of claim [27] 23 which contains *ery* modules [1-5] 1 through 5 inclusive and narbomycin module 6, wherein said polyketide chain is transferred from *ery* modules [1-5] 1 through 5 inclusive to *nar* module 6.

30. (Amended) The hybrid modular polyketide PKS of claim [27] 23 which contains modules 1 and [3-6] 3 through 6 inclusive of *ery* and modules 2-3 of tylosin, spiramycin or niddamycin, wherein said polyketide chain is transferred from *ery* module 1 to modules 2-3 of tylosin, spiramycin or niddamycin and then to *ery* modules [3-6] 3 through 6 inclusive.

31. (Amended) The hybrid modular polyketide PKS of claim [27] 23 which contains modules [1-3] 1 through 3 inclusive of tylosin, spiramycin or niddamycin and modules [3-6] 3 through 6 inclusive of *ery*, and wherein said polyketide chain is transferred from modules [1-3] 1 through 3 inclusive of said tylosin, spiramycin or niddamycin to *ery* modules [3-6] 3 through 6 inclusive.

32. (Amended) The hybrid modular polyketide PKS of claim [27] 23 which contains a module of tylosin, spiramycin or niddamycin and modules 1-2 and [3-6] 3 through 6 inclusive of *ery*, wherein said polyketide chain is transferred from *ery* modules 1-2 to the tylosin, spiramycin or niddamycin module and then to *ery* modules [3-6] 3 through 6 inclusive.

33. (Amended) The hybrid modular polyketide PKS of claim [27] 23 which contains modules 1 and [3-6] 3 through 6 inclusive of *ery* and module 5 of tylosin, spiramycin or niddamycin having the enoyl reductase catalytic activity inactivated, wherein said polyketide chain is transferred from *ery* module 1 to module 5 of tylosin, spiramycin or niddamycin and then to *ery* modules [3-6] 3 through 6 inclusive.

34. (Amended) The hybrid modular polyketide PKS of claim [27] 23 which contains *ery* modules [1-4] 1 through 4 inclusive and 6 and module 6 of spiramycin or niddamycin, wherein said polyketide chain is transferred from *ery* modules [1-4] 1 through 4 inclusive to module 6 of spiramycin or niddamycin and then to *ery* module 6.

35. (Amended) The hybrid modular polyketide PKS of claim [27] 23 which contains module 1 of FK-506 or 520 and modules [2-14] 2 through 14 inclusive of rapamycin, wherein said polyketide chain is transferred from module 1 of FK-506 or 520 and then to modules [2-14] 2 through 14 inclusive of rapamycin.

36. (Amended) The hybrid modular polyketide PKS of claim [27] 23 which contains module 1 and [11-14] 11 through 14 inclusive of rapamycin and modules [2-6] 2 through 6 inclusive of FK-506 or 520 wherein said polyketide chain is transferred from module 1 of rapamycin to modules [2-6] 2 through 6 inclusive of FK-506 or 520 and then to modules [11-14] 11 through 14 inclusive of rapamycin.

37. (Amended) The hybrid modular polyketide PKS of claim [27] 23 which contains module 1 of rapamycin, modules [2-7] 2 through 7 inclusive of FK-506 or 520 and modules [12-14] 12 through 14 inclusive of rapamycin, wherein said polyketide chain is transferred from module 1 of rapamycin to modules [2-7] 2 through 7 inclusive of FK-506 or 520 and then to modules [12-14] 12 through 14 inclusive of rapamycin.

38. (Amended) The hybrid modular polyketide PKS of claim [27] 23 which contains module 1 of rapamycin, modules [2-8] 2 through 8 inclusive of FK-506 or 520 and modules 13-14 of rapamycin, wherein said polyketide chain is transferred from module 1 of rapamycin to modules [2-8] 2 through 8 inclusive of FK-506 or 520 and then to modules 13-14 of rapamycin.

39. (Amended) The hybrid modular polyketide PKS of claim [27] 23 which contains modules [1-10] 1 through 10 inclusive of rapamycin and modules [7-10] 7 through 10 inclusive of FK-506 or 520, wherein said polyketide chain is transferred from modules [1-10] 1 through 10 inclusive of rapamycin to modules [7-10] 7 through 10 inclusive of FK-506 or 520.